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Characterization of physico-chemical properties and antioxidant activity of oil from seed, leaf and stem of purslane *(Portulaca oleracea* L.*)*

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ABSTRACT

Purslane *(Portulaca oleracea* L.*)* is a member of the family of portulacaceae and is a very good source of alpha-linoleic acid. Alpha-linoleic is an omega-3 fatty acid which plays an important role in human growth, development and in preventing diseases. Characterization of the physicochemical properties and antioxidant activity of oil from seeds, leaves and stems of purslane, and appraisal of its viability is the main purpose of this study. In this work, physicochemical properties of oil from seed, leaf and stem of purslane were characterized and the highest oil content was observed in seed (11.25 %) using solvent extraction method. The highest specific gravity was recorded for seed oil (1.10 w/w) followed by leaf oil (0.73 w/w) with non-significant difference. Moreover, oil extracted from purslane stem was found to be the least in oil content (2 %) and specific gravity (0.14 w/w); but the highest in acid value (13.32 w/v) and percentage free fatty acid. The peroxide value was found to be the highest for seed oil and the least for stem with significant difference. The highest ascorbic acid content was recorded for purslane seed oil (41.67 %) followed by leaf oil (32.29 %). Likewise, the highest 1, 1- diphenyl-2-picrylhydrazine activity was obtained for leaf oil (12.55 %) followed by seed oil (2.05 %). The plot for PC2 vs PC1 showed the oil content, specific gravity, peroxide value, ascorbic acid and 1, 1- diphenyl-2-picrylhydrazine having close PC1 and PC2 scores with vector angle $< 90^0$ showing correlated effects. Characterizing physical and chemical properties and antioxidant activity of oil from seeds, leaves and stems of purslane is crucial in providing necessary information for the utilization of the oil content.

1. Introduction

Portulaca oleracea L. commonly known as purslane is a herbaceous weed belongs to the family of Portulacaceae. Purslane is found all over the world including the temperate countries; most abundant in India and Mediterranean basin [1]. It can be found growing in almost any unshaded area, including flower beds, corn fields, and waste places. It is naturally existing as a weed in field crops and lawns. Purslane is an important component of green salad and as vegetable juice [2]. Its medicinal value is evident from its use for treatment of pain and edema. Purslane has strong regenerative effects on a cellular level and is loaded with antioxidants, beta carotene, vitamin C, and vitamin E, and also contains a high amount of the omega-3 fatty acids, which helps to reduce wrinkles [3]. All these ingredients are powerful antioxidants that can help to brighten the complexion and even prevent future signs of aging by reducing UV-induced damage [4]. Purslane is a key to a great anti-ageing skin care regimen and reduce inflammation in the skin, improve overall blood flow, and stimulate cell repair, which decreases the appearance of scars and wrinkles [5]. This plant is also pharmacologically studied for its anti-fungal, anti-inflammatory, anti-oxidant, anti-microbial and wound healing properties [6]. It also reported to contain other chemical constituents, including urea, calcium, iron, phosphorous, manganese, copper and fatty acids, especially omega-3-acids whose concentration in purslane is the highest found in leafy vegetables [7]. Furthermore, the occurrence of glutathione; glutamic acid; and aspartic acid has been reported by Simopoulos [8]. Recent research has shown that purslane is a rich source of omega-3 (ω-3) fatty acids [9], which is important in preventing heart attacks and strengthening the immune system [10]. In addition, purslane is reported to be rich in linolenic acid and ß-carotene and it's used as a healthy food [11] for patients with cardiovascular diseases [12]. Purslane is a very good source of α-linolenic acid which is an omega-3(ω-3) fatty acid that can't have

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synthesized in our body and play an important role in human growth and development and in preventing diseases [12]. Purslane has received renewed interest since the identification of some of its nutritional and medicinal properties [13]. In the last few decades there has been an increasing interest in the ethno pharmacological studies on medicinal plants, which is evident by numerous publications and reports [14]. However, these reports on medicinal plants are widely scattered in journals and books pertaining to different disciplines. Purslane obviously found as a weed in field crops and lawns, however, little is known about its folklore medicine and nutritional diet. Therefore, analyzing of the physicochemical properties and antioxidant activity of oil from seeds, leaves and stems of purslane will provide valuable information to increase societal knowledge on its medical and dietary importance.

2. Materials and methods

2.1. Chemicals and materials

All reagents and solvents used in this study were of analytical grade. The raw samples of purslane seeds, leaves and stem were collected from Dire Dawa administration region, Ethiopia where naturally growing as a wild weed. $C_{20}H_{14}O_4$, Glacial Acetic acid, Chloroform, HPO_3, H_2O_2, Methanol, Petroleum Ether, and hexane analytical grade and Flask, Water bath, Oven, Centrifuge (Model, Z300, 580 W, 3052 Nm, German) soxhlet apparatus were also used.

2.2. Plant material and extract preparation

Purslane (*Portulaca oleracea* L) was collected from Dire Dawa administration region in Ethiopia where naturally growing actually as a wild weed. The authenticity of the plant material was confirmed at the Herbarium of Haramaya University, Ethiopia. Purslane seeds were obtained via collection of the ripe top of the cone seed pods before the seeds scatters. The fresh samples manually washed with distilled water and residual moisture were evaporated at room temperature.

The leaf and stem samples were cut into pieces then dried in oven at 40 °C for 72 h. Then ground to a fine powder in a grinder for 2 min, at 15 s. intervals, the process was stopped for 15 s. to avoid heating of sample. The extracts were determined using the standard methods of the Association of the Analytical Chemists [15]. Determination of moisture 5 % was carried out [16].

2.3. Extraction and determination of oil content

The leaf and stem samples were oven dried at 40 °C and then ground to fine powder. The seed was separately treated at modest temperature to avoid bioactive change in DPPH, Vitamin C and fatty acid. Six flasks sterilized in hot air oven were used. The extraction of oil from purslane leaf, stem & seed by dissolving in petroleum Ether using soxhlet apparatus. 20 g of leaf, stem and seed samples were mixed with petroleum ether kept in soxhlet apparatus for 8 h. The crude extract was concentrated by using rotate evaporator by adding sodium sulfate. The physicochemical characterization of the % oil yield of the sample was determined as: Oil content = $\frac{\text{oil weight(OW)}}{\text{sample weight(SW)}}$ x 100 where oil weight = W2-W1: W1= Weight of the extraction flask (g); W2=Weight of the extraction flask plus the dried crude fat (g). The specific gravity of the oil was determined gravimetrically by employing the weight ratio of the oil to the equivalent amount of water according to the following formula: Specific gravity = $\frac{W2}{W1}$ Where, W2 and W1 are the weights of oil and equivalent amount of water respectively.

2.4. Determination of acid value

The acid value was determined stated standard methods of the Association of the Analytical Chemists [16]. Two gram of oil sample was weighted into a 250 mL conical flask. 25 mL diethyl ether was mixed with 25 mL alcohol and 1 mL of 1 % phenolphthalein indicator and added to the oil sample. The conical flask was then placed on a hot water bath until the oil completely dissolved in the solvent. The hot solution was titrated with 0.1 M KOH until a pink colour which persisted for 15 s noticed. The acid value was calculated as: Acid value = $\frac{\text{Titre(ml)x 5.61}}{\text{Weight of sample used}}$. Estimation of free fatty acid, the percentage free fatty acid was estimated by multiplying the acid value with the factor 0.503. The % FFA = 0.503×Acid value [17].

2.5. Determination of peroxide value

The peroxide value was determined as described standard methods of the Association of the Analytical Chemists [15]. One gram of the oil sample was weighed and added into a clean boiling tube. 1 g of powdered potassium iodide and 20 mL of solvent mixture (2 volumes glacial acetic acid + 1 vol chloroform) was added to the hot oil sample. The tube was placed in boiling water and left for 10 min to boil vigorously. The content of the boiling tube was then poured into a flask containing 20 mL of 5 % potassium iodide solution, the tube was washed out twice with 25 mL of water and then titration with 0.002 M sodium thiosulphate solution was done using starch as an indicator. A blank test was carried out at the same time under same condition without the oil. The peroxide values of the samples were calculated using equation [18]. Peroxide value = $\frac{\text{T x M x 100}}{\text{Weight of the sample}}$ Where, T = Titre value; M = molarity of $Na_2S_2O_3$. The peroxide value was reported as the number of ml of 0.0002 m sodium thiosulphate per gram of sample. The value gotten was multiplied by 2 which equaled milli equivalent of peroxide oxygen per kg of sample (meq/kg). PV = 2XV, where: PV = Peroxide value, V = Volume of Sodium thiosulphate used, 2 = (N x 1000) / W, N = Normality of Sodium thiosulphate used, W = Weight of sample used.

2.6. DPPH radical scavenging activity

The radical scavenging activity of the oil extract was adopted to measure antioxidant activity using the 1,1-diphenyl-2-picrylhydrazine (DPPH) method [19]. Briefly, 2 mL of extract solution (1–100 μg/mL) in ethanol was added to 2 mL of DPPH (0.1 mM) solution. The mixtures were kept aside in a dark area for 30 min and absorbance was measured at λ_{max}517 nm against an equal amount of DPPH and methanol as a blank. The percentage of DPPH radical scavenging activity (RSA%) was estimated using the equation:

DPPH radical scanveging activity(%) = $\frac{(A_{control} - A_{sample})}{A_{control}}$x100, Where A 0 is the absorbance of the control and A1 is the absorbance in the presence of the sample. Ascorbic acid will be used as positive control.

2.7. Hydrogen peroxide scavenging activity

The radical scavenging activity of individual oil extracts was determined using the H_2O_2 method [20]. Briefly, 2 mL of extract solution (10−100 μg/mL) in methanol was added to 4.0 mL of H_2O_2 20 mM solution in phosphate buffer (pH 7.4). After 10 min, the absorbance was measured at λ_{max} 230 nm against the phosphate buffer blank solution. The percentage scavenging of H_2O_2 was calculated using the equation: % scavenging of H_2O_2 = [(A0 -A1)/

A0] x100, Where A0 = absorbance of the control (phosphate buffer with H_2O_2) and A1 = absorbance of the test extracts.

2.8. Ascorbic acid analysis

The ascorbic acid content of oil was determined by the 2, 6-dichlorophenol indophenol dye method [16]. 5 mL of the standard ascorbic acid solution was pipetted into a 100 mL conical flask and 5 mL of the 3 % HPO_3 solution was added. The ascorbic acid solution was titrated with the dye solution to a pink color, would persist 15 s. The titre value was recorded. The dye factor was calculated by dividing 5 mL volume of ascorbic acid solution taken for titration by titrant volume of dye solution. Dye factor was expressed as mg of ascorbic acid per ml of the dye. Since 5 mL of the standard ascorbic acid solution contains 0.5 mg ascorbic acid: Dye factor (mg ascorbic acid per dye) = $\frac{0.5mg}{titrant\ volume}$, 1 mL of extract oil was diluted to 5 mL with 3 % meta phosphoric acid in a 50 mL volumetric flask. Then the aliquot was centrifuged (Model, Z300, 580 W, 3052 Nm, German) for 15 min and titrated with the standard dye to a pink end point (persisting for 15 s). The ascorbic acid content was calculated from the titration value, dye factor, dilution and volume of the sample as % A. $A = \frac{(ABRsample)x\ dye\ factor\ x\ volume\ of\ initial\ test\ solution}{volume\ of\ test\ solution\ titrated}$ x 100%, Where: A. A = Ascorbic Acid; ABR = Average Burette reading.

2.9. Statistical analysis

Statistical analysis was carried out using SPSS software version 20 for windows. The means ± SD for triplicate assays of all parameters were examined for significance using ANOVA with *t*-test to determine any significant difference between the treatments at $P < 0.05$.

3. Results

Physicochemical properties of oil from seed, leaf and stem of purslane indicated in Table 1. The highest oil content was observed in seed (11.25 %) using solvent extraction method. The leaf found to contain higher oil content than stem, with significant difference. The highest specific gravity was recorded for seed oil (1.10 w/w) followed by leaf oil (0.73 w/w) with non-significant difference. Considerably oil extracted form purslane stem was found to be the least in oil content (2 %) and specific gravity (0.14 w/w); however, the highest in acid value (13.32 w/v) and percentage free fatty acid (% FFA). The peroxide value was found to be the highest for seed oil but the least for stem oil with significant difference.

Acid Value (AV) and Free Fatty Acid (FFA) are analytically used to detect the level of unesterified fatty acid in a lipid sample to define its quality. Both the AV and FFA are used to estimate the amount of oil that will be lost during refining steps designed to remove fatty acid. Hence, high acidity level means a poorly refined oil or fat breakdown after storage or use. If the fatty acid librated is volatile, acid value or free fatty acid may be a measure of hydrolytic rancidity. Accordingly, in the present study, the least acid value of 2.81 mg KOH / g of oil for the purslane leaf sample and 13.32 mg KOH / g of oil for purslane stem sample indicate respective high level of long chain carboxylic acids in the purslane oil samples. Antioxidant activities of oil from seed, leaf and stem purslane as indicated in Table 2. In this study, two in vitro tests, DPPH and hydrogen peroxide scavenging activities, were used to screen the radical scavenging activity of oil extracted from purslane. The different methods for measuring the radical scavenging potential can give different results according to which specific free radical is being used as a reactant. DPPH is often used to test how far compounds can act as free radical scavengers or hydrogen donors, and to quantify antioxidants in complex systems. Antioxidants may be reductants and inactivation of oxidants by reductants are redox reactions in which one reaction species is reduced when the other is oxidized. The DPPH scavenging ability of purslane seed oil was enhanced when the oil concentration was increased. A strong linear relationship is observed within the range of purslane seed oil concentrations from 3−20 mg/mL. Moreover, it is worth mentioning that purslane seed oil antioxidant activity could only be detected at concentrations at or above 3 mg/mL.

Table 2
Antioxidant activities of purslane oil.

Plant organ	Ascorbic acid	Peroxide scavenging activity	DPPH
Leaf	32.29 ± 3.93ab	0.35 ± 0.14a	12.55 ± 0.07a
Seed	41.67 ± 3.90a	0.45 ± 0.07a	2.05 ± 0.35b
Stem	25.00 ± 1.47b	0.70 ± 0.07a	0.35 ± 0.13b

In this study, the highest ascorbic acid content was recorded for purslane seed oil (41.67 %) followed by leaf oil (32.29 %). Likewise, the highest DPPH activity was obtained for leaf oil (12.55 %) followed by seed oil (2.05 %). However, the peroxide scavenging indicated to be the highest for stem oil (0.70 %) and the least for leaf oil (0.35 %) insignificantly. These indicate that leaf and seed oils have shown more quality than stem oil. The first principal component retained information contained in 46.16 % of the original variables while the second component retained only 1.36 % of the original variable. Thus these two components having Eigen value greater than one, accounting for 96 % of the variable suggesting that these principal component analyses might be sufficient to explain the relationship between variables in the original data. The principal component analysis in the present study, was shown that the first component has got high negative loading from acid value, free fatty acid and hydrogen peroxide scavenging activity while high positive component loadings were obtained for oil content, specific gravity, peroxide value, ascorbic acid and DPPH. Factors that load high positive or negative loading have showing opposite effects. Based on the plot for PC2 vs PC1 statistics, oil content, specific gravity, peroxide value, ascorbic acid and DPPH having close PC1 and PC2 scores of vector angle < 90° showing correlated effects while these parameters with HPSC and free fatty acid have vector angle greater than 90^0 showing opposite effects or more divergence. Furthermore, HPSC, free fatty acid and acid value have similar effect since their vector angle < 90^0, that is the lower amount of HPSC, free fatty acid and acid value the more the oil quality while the oil content, specific gravity, peroxide value, ascorbic acid and DPPH, the higher the oil quality.

4. Discussion

Purslane is best used for human consumption as green vegetable rich in minerals and omega-3 fatty acids [21]. Omega-3 fatty acid is a

Table 1
Physicochemical properties of oil from seed, leaf and stem of purslane.

Plant organ	oil content (%)	Specific gravity (w/w)	Acid value (w/v)	FFA (%)	Peroxide value (meq/kg)
Seed	11.25 ± 1.77a	1.10 ± 0.14a	4.21 ± 1.97b	2.12 ± 1.99b	0.80 ± 0.14a
Leaf	6.25 ± 1.77b	0.73 ± 0.15a	2.81 ± 1.98b	1.41 ± 0.99b	0.75 ± 0.21a
Stem	2.00 ± 0.71c	0.14 ± 0.04b	13.32 ± 0.99a	6.70 ± 0.50a	0.40 ± 0.14b

precursor of a specific group of hormones. As stated by Milwidsky [22] fats and oils are triglycerides, the free fatty acids should be very low in highly graded lipid sample. In addition to free fatty acids, acids phosphates and amino acids can also contribute to acidity. Free fatty acid is the percentage by weight of a specified fatty acid or oleic or lauric acid. For fatty acids, the acid value, in conjunction with the saponification value, can be used to give a measure of the amount of neutral fat present. The free fatty acid is normally expressed as oleic acids. The exceptions are coconut and palm kernel oils, which are calculated as lauric acids [23]. All the examined methods of drying significantly lowered the antioxidant capacity of the sample. Analysis of data demonstrated that among investigated dried sample, hot air dried and freeze-dried purslane leaves retained a better antioxidant capacity independently by the temperature applied while microwave procedure drastically reduced the antioxidant potential of related to the different phytochemical content in investigated samples [24]. However, the generation and accumulation of antioxidants during food dehydration may cause antagonistic or synergistic effects with each other or with other compounds present in the sample. Similarly, recent studies show that, the content in linolenic acid, linoleic acid and oleic acid is strongly influenced by the percentage of substitution. In fact, the initial content of FAMEs detected in dry purslane influenced the final fatty acid content of the fortified food [25]. The complex interactions influencing the functional properties of food during drying require further research [26]. An increase in antioxidant activity is one of the main aims of food supplementation. Several assays highlighted the capacity of purslane to increase the antioxidant potential of enriched samples [25]

The methanol extracts edible fresh parts of thirteen purslanes were similarly examined for their phytochemical content and antioxidant activity by using the DPPH radical scavenging method and Ferric reducing antioxidant power assay [27]. Peroxide value (PV) measures the degree of lipid oxidation in fats and oils but not their stability. Peroxide value measures a transient product of oxidation. A low value may represent early or advanced oxidation; which can be distinguished with time. The PV in purslane oil gave the highest value for purslane seed and the least value for oil extracted from stem [28]. The half maximal inhibitory concentration (IC50) values ranged from 2.52 to 3.29 mg/mL for DPPH test, and for 7.39–104.2 μmol TE/g DW for Ferric reducing antioxidant power assay. Differently, similar DPPH radical scavenging results were obtained with air-dried powered purslane. In fact, the *n*-hexane, dichloromethane, chloroform, ethyl acetate and methanol extracts showed IC50 values in the range from 62.9–91.0.8 mg/mL for ethyl acetate and dichloromethane extracts, respectively [29]. The IC50 values of DPPH radicals scavenging of purslane seed oil were 11.16 ± 0.07075 mg/mL respectively [30]. Additionally, the DPPH free radical scavenging capacity of purslane seed oil was higher than that of walnut oil (IC50 147.0 mg/mL) [31] and weaker than that of flaxseed oil (IC50 3.31 mg/mL) [32]. The main fatty acid components of flaxseed oil are alpha-linolenic (41.22 %), linoleic (15.44 %), and oleic (28.2 %) acids. Obviously, the stronger DPPH radical scavenging ability of purslane seed oil may be due to its higher content of linolenic omega-3 fatty acid [30]. Likewise, purslane is very good source of alpha-linolenic acid and gamma-linolenic acid (LNA, 18: 3 w3) (4 mg/g fresh weight) of any green leafy vegetable. It contained the highest amount (22.2 mg and 130 mg per 100 *g* of fresh and dry weight, of alpha-tocopherol and ascorbic acid (26.6 mg and 506 mg per 100 *g* of fresh and dry weight, respectively [25]. The fresh purslane leaf ethanolic extract exerted the greatest DPPH radical scavenging activity with IC50 value of 52.86 mg/ ml [30]. This extract was followed by fresh leaf hydroalcoholic extract (IC50 value of 53.92 mg/mL). The principal component analysis in the present study, was shown that the first component has got high negative loading from acid value, free fatty acid and hydrogen peroxide scavenging activity while high positive component loadings were obtained for oil content, specific gravity, peroxide value, ascorbic acid and DPPH.

5. Conclusion

Characterization of physicochemical properties of oil from seed, leaf and stem of purslane suggests that the highest oil content was observed in seed using solvent extraction method. It is relatively obvious from this study result that leaf found to contain higher oil content than stem. The lower amount of HPSC, free fatty acid and acid value the more the oil quality and higher progressive component were gained for oil content, specific gravity, peroxide value, DPPH and ascorbic acid.

Authors' contributions

MD and ZY outset and designed experiments. MD, AM and ZY performed experiments. MD and ZY contributed reagents and materials. MD and AM performed all the experiment and data analyses and wrote the manuscript. All authors discussed and revised the manuscript. All authors commented on the manuscript before submission. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare they have no competing interests.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2020.e00512.

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